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(54) Title: METHOD OF INACTIVATION OF VIRAL AND BACTERIAL BLOOD CONTAMINANTS

(57) Abstract

A method is provided for inactivating viral and/or bacterial contamination in blood cellular matter, such as erythrocytes and platelets, or protein fractions. The cells or protein fractions are mixed with chemical sensitizers and irradiated with, for example, gamma or X-ray radiation.

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METHOD OF INACTIVATION OF VIRAL AND BACTERIAL BLOOD CONTAMINANTS

FIELD OF THE INVENTION

This invention relates to the general field of biochemistry and medical sciences, and specifically to inactivating viral/bacterial contamination of lyophilized or reconstituted blood cell compositions comprising erythrocytes, platelets, etc, or protein fractions.

BACKGROUND OF THE INVENTION

A major concern in the use of stored or donated homologous blood or plasma protein preparations derived from human blood is the possibility of viral and bacterial contamination.

Viral inactivation by stringent sterilization is not acceptable since this could also destroy the functional components of the blood, particularly the erythrocytes (red blood cells) and the labile plasma proteins. Viable RBC's can be characterized by one or more of the following: capability of synthesizing ATP; cell morphology; P_{50} values; oxyhemoglobin, methemoglobin and hemichrome values; MCV, MCH, and MCHC values; cell enzyme activity; and *in vivo* survival. Thus, if lyophilized then reconstituted

and virally inactivated cells are damaged to the extent that the cells are not capable of metabolizing or synthesizing ATP, or the cell circulation is compromised, then their utility in transfusion medicine is compromised.

There is an immediate need to develop protocols for the deactivation of viruses that can be present in the human red blood supply. For example, only recently has a test been developed for Non A, Non B hepatitis, but such screening methods, while reducing the incidence of viral transmission, do not make the blood supply completely safe or virus free. Current statistics indicate that the transfusion risk per unit of transfused blood is as high as 1:100 for Non A, Non B hepatitis, and ranges from 1:40,000 to 1:1,000,000 for HIV, depending on geographic location. Clearly, it is desirable to develop a method which inactivates or removes virus indiscriminately from the blood.

Contamination problems also exist for blood plasma protein fractions, such as plasma fractions containing immune globulins and clotting factors. For example, new cases of non A, non B hepatitis have occurred in hemophilia patients receiving protein fractions containing Factor VIII which have been treated for viral inactivation according to approved methods. Therefore, there is a need for improved viral inactivation treatment of blood protein fractions.

The present invention thus provides a method for the inactivation of viral and bacterial contaminants present in blood and blood protein fractions.

SUMMARY OF THE INVENTION

The present invention provides a method for viral/bacterial inactivation of dried or reconstituted cells (erythrocytes, platelets, hemosomes and other cellular or cell-like components) or blood protein fractions, which allows for the cells or protein fractions to be useful in a transfusable state, while still maintaining relatively high cell viability, ATP synthesis and oxygen transport, in the case of cellular components, and therapeutic efficacy, in the case of protein fractions.

The lyophilization and reconstitution media according to the present invention may be utilized to lyophilize and reconstitute proteins, particularly, blood plasma protein fractions. The protein fraction may be virally/bacterially deactivated by mixing with a chemical sensitizer, lyophilized (freeze-dried), then irradiated. If the lyophilization media of the invention is used, it is contemplated that the constituents of the media also serve to provide some degree of protection of the dry proteins during irradiation.

A preferred embodiment comprises reducing viral and bacterial contamination of dried or reconstituted cells with washing solutions containing a polymer or mixture of polymers having a molecular weight in the range of about 1K to 360 K, followed by one or more additional wash cycles using a wash of a dextrose-saline solution at a pH in the range of about 7.0-7.4. The dextrose-saline solution will also contain a polymer having a molecular weight in the range of about 1K to 40K, and preferably about 2.5K.

The composition of reconstituted cells will also preferably contain a monosaccharide.

Preferably the cells will have been previously lyophilized using a lyophilization solution buffered in the range of pH of 7.0 to 7.4 preferably by a phosphate-buffered solution. A typical phosphate-buffered lyophilization solution will comprise mono- and di-basic potassium and sodium phosphate (usually in the range of 1-10 mM each) and 5-10 mM adenine. This solution maintains the pH at around 7.2.

A preferred phosphate-buffered solution to be used as the lyophilization buffer will comprise nicotinic acid, reduced glutathione, glutamine, inosine, adenine, monopotassium phosphate, magnesium chloride disodium phosphate all of which will serve as a basic salt buffer at a pH of about 7.2. In addition this lyophilization buffer will contain a final concentration of about 26% weight by volume of a monosaccharide, preferably 1.7 M glucose, and a final concentration of about 3.0% weight by volume of polyvinylpyrrolidone (average molecular weight of 360K), and a final concentration of about 15% weight by volume of hydroxyethyl starch (average molecular weight of 500K).

The term lyophilization is broadly defined as freezing a substance and then reducing the concentration of the solvent, namely water, by sublimation and desorption, to levels which will no longer support biological or chemical reactions. Usually, the drying step is accomplished in a high vacuum. However, with respect to the storage of cells and particularly erythrocytes, the extent of

drying (the amount of residual moisture) is of critical importance in the ability of cells to withstand long-term storage at room temperature. Using the procedure described herein, cells may be lyophilized to a residual water content of less than 10 weight %, preferably less than 3%, and still be reconstituted to transfusable, therapeutically useful cells. Cells with about 3 weight % water content using this procedure may be stored for up to two weeks at room temperature, and at 4°C for longer than eight months, without decomposition. This far exceeds the current A.A.B.B. standard for refrigerated storage of red blood cells of six weeks at 4°C or less than one day at room temperature without decomposition. These dried cells may be deactivated using a chemical sensitizer described herein.

According to the preferred embodiment of the present invention the washed packed red blood cells are mixed with a chemical sensitizer, then washed to remove excess sensitizer not bound to viral or bacterial nucleic acid, and the treated cells are then lyophilized. The dry cell and sensitizer mixture will then be irradiated, typically with gamma radiation, at an intensity of about 3K-50K rads, for a period of time sufficient to destroy viruses (in particular, the single-stranded or double-stranded RNA/DNA viruses), without any substantial adverse effect on the recovery and usefulness of the cells. Other wavelengths of electromagnetic radiation such as X-rays, may be used.

In another preferred embodiment, the chemical sensitizers may be added to liquid protein

preparations, then lyophilized and irradiated. Particularly preferred are blood protein preparations, including but not limited to, plasm proteins, blood protein extracts, clotting factor (such as Factors VIII and IX), immune globulins a serum albumin.

Dry (lyophilized) cells or protein fractions may directly mixed with the chemical sensitizer, then irradiated.

From the foregoing description, it will be realize that the invention can be used to selectively binc metal atom or a metal atom containing chemical sensitizer to blood-transmitted viruses, bacteria, parasites. Also monoclonal or polyclonal antibodi directed against specific viral antigens (either c proteins or envelope proteins) may be covalently coupled with either a metal atom or a metal atom-containing sensitizer compound, thereby increasing the effective cross-section of the contaminant to penetrating or other forms of radiation energy.

Since cell compositions also comprise a variety of proteins, the method of decontamination of cells described herein is also applicable to protein fractions, particularly blood plasma protein fractions, including, but not limited to, fractions containing clotting factors (such as Factor VIII anc Factor IX), serum albumin and/or immune globulins. The viral and bacterial inactivation may be accomplished by treating a protein fraction with a sensitizer as described herein. A protein fraction which has been lyophilized and reconstituted may be sensitized and irradiated to deactivate possible

contamination. It is contemplated that liquid and frozen protein fractions may also be decontaminated according to the present invention.

Depending upon the nature of the presumed radiolytic mechanism of the sensitizer reaction with the virus, other types of radiation may be used, such as X-ray, provided the intensity and power utilized is sufficient to inactivate the viral contamination without adverse effect on the cells. Mature human red blood cells and platelets lack nucleic acids; therefore the nucleic acid binding sensitizers selectively target contaminating viruses and bacteria. Although described in connection with viruses, it will be understood that the methods of the present invention are generally also useful to any biological contaminant found in stored blood or blood products, including bacteria and blood-transmitted parasites.

DETAILED DESCRIPTION OF THE INVENTION

The cells are preferably prepared by immersing a plurality of erythrocytes, platelets and/or hemosomes, etc. in a physiologic buffered aqueous solution containing a carbohydrate, and one or more biologically compatible polymers, preferably having amphipathic properties. By the term amphipathic it is meant there are hydrophobic and hydrophilic portions on a single molecule. This immersion is followed by freezing the solution, and drying the frozen solution to yield novel freeze-dried erythrocytes containing less than 10%, and preferably about 3% or less by weight of moisture, which, when reconstituted, produce a significant percentage of viable, transfusably useful red blood cells,

platelets or hemosomes. Preferred methods of reconstitution of the lyophilized composition are described below. Although described in connection with red blood cells, it will be understood that the methods are generally also useful to lyophilize platelets, hemosomes, and blood protein fractions.

The carbohydrate utilized to prepare erythrocyte, platelet and/ or hemosome compositions according to the invention is biologically compatible with the erythrocytes, platelets or hemosomes, that is, non-disruptive to the cells or hemosome membrane, and one which permeates, or is capable of permeating, the membrane of the erythrocytes, platelets or hemosomes. It is also advantageous to stabilize proteins, especially labile blood proteins, with the carbohydrates during lyophilization and irradiation according to the invention. The carbohydrate may be selected from the group consisting of monosaccharides, since disaccharides do not appear to permeate the membrane to any significant extent. Monosaccharide pentoses and hexoses are preferred as is a final concentration of from about 7.0 to 37.5 weight % in phosphate buffered saline (PBS) or a phosphate buffered solution, preferably about 26%. Xylose, glucose, ribose, mannose and fructose are employed to particular advantage.

It will be understood that the cells may be lyophilized using other protocols and irradiated as described below. Although viral inactivation will be attained, the advantage of retaining a significant percentage of viable useful red blood cells is lost if the described lyophilization procedure is not followed.

The invention will be hereafter described in connection with erythrocytes (RBC's) but it will be understood it is also applicable to platelets, hemosomes or other blood cell types or biological cells, as well as protein fractions, particularly plasma protein fractions.

The erythrocytes will preferably be prepared from whole blood centrifugation, removal of the plasma supernatant and resuspending the cells in PBS or a phosphate buffered solution or a commercial dextrose-saline solution. This wash cycle may be repeated 2-3 times preferably using a commercial dextrose-saline solution, then the packed cells are diluted with the lyophilization buffer described above so that the final diluted concentration of carbohydrate and polymer are maintained in the necessary ranges.

Alternatively, commercially available packed blood cells may be used, which typically are prepared in CPDA (commercial solution containing citrate, phosphate, dextrose and adenine).

Upon lyophilization to a moisture content of less than 10%, and preferably less than 3%, the lyophilized cells may be maintained under vacuum in vacuum-tight containers, or under nitrogen or other inert gas, at room temperatures for extended periods of time in absence of or without significant degradation of their desirable properties when reconstituted for use as transfusable cells. In using the preferred lyophilization method disclosed herein, a particular advantage of the present invention is that the lyophilized cells may be stored at room temperature for extended periods of time,

thus obviating the need for low temperature refrigeration which is required for storing liquid CPDA preserved red blood cells prepared by methods of the prior art. The present invention also obviates the need for very low temperature (-80°C) frozen storage of red blood cells in glycerol.

By using the preferred reconstitution method disclosed herein it is a further advantage that the lyophilized red blood cells may be reconstituted at normal temperatures, i.e. greater than about 4°C up to about 37°C, which corresponds to normal human body temperature, and preferably at room temperature (about 22°C). The reconstitution medium is preferably a solution comprising a polymer or mixture of polymers having a molecular weight of from about 2.5K to 360 K, preferably 5K to about 360K, present in a concentration in the range of about 12 to 30% weight by volume. This polymer may be the same polymer utilized to lyophilize the red blood cells as described above. Hence the polymers polyvinylpyrrolidone, hydroxyethyl starch, and dextran are particularly preferred and most preferred is polyvinylpyrrolidone (preferably molecular weight about 10K) present in a concentration of about 19% weight by volume in the reconstitution solution. The reconstitution solution will be buffered again typically by phosphate-buffered solution comprising monopotassium phosphate and disodium phosphate as described hereinabove to maintain a pH within the range of about 7.0 to 7.4. The most particularly preferred polymer is polyvinylpyrrolidone of an average molecular weight of about 10K. The most preferred reconstitution buffer will also contain

adenosine triphosphate (ATP) in a final concentration of about 5mM.

The polymers may be present in the various solutions from a final concentration of about 3.6% weight % up to saturation, and have a molecular weight in the range of from about 2.5K to about 360K. Preferably, the polymers have molecular weights in the range of from about 2.5K to about 500K, most preferably from about 2.5K to 50K, and are present in a concentration of from about 3.6 weight % up to the limit of solubility of the polymer in the solution. Polymers selected from the group consisting of polyvinylpyrrolidone (PVP) and polyvinylpyrrolidone derivatives, and dextran and dextran derivatives provide significant advantages. Most preferred is the use of polyvinylpyrrolidone (an amphipathic polymer) of average molecular weight in the range of 2.5-360K in an amount in the range of 3-20% weight by volume in the solution prior to lyophilization. Amino acid based polymers (i.e., proteins), dextrans or hydroxyethyl starch may also be employed. In the lyophilization buffer hydroxyethyl starch (M-HES) with an average molecular weight of about 500K is employed in a 15% weight by volume final concentration. Other amphipathic polymers may be used, such as poloxamers in any of their various forms. The use of the carbohydrate-polymer solution in the lyophilization of red blood cells allows for the recovery of intact cells, a significant percentage of which contain biologically-active hemoglobin.

The most preferred reconstitution buffer will be a solution comprising monopotassium phosphate, disodium

phosphate and ATP, all of which form a basic salt buffer at a pH of about 7.2, which also contains about 19% weight by volume of polyvinylpyrrolidone (average molecular weight about 10K).

The reconstitution solution may also optionally contain a monosaccharide, preferably present in the concentration range of about 7.0 to 37.5% weight by volume. The preferred monosaccharides are xylose, glucose, ribose, mannose and fructose.

In the most preferred embodiment, the lyophilized erythrocytes can be reconstituted by mixing with an equal volume of the reconstitution buffer at a temperature of about 37°C and mixed. By "equal" it is meant that the volume is the same as the starting volume prior to lyophilization. After initial reconstitution, the solution is preferably diluted 1:1 with 1-4 additional volumes of the reconstitution buffer at a temperature of about 37°C with added mixing until fully hydrated.

Then, it is preferred that the rehydrated cells be washed according to the following procedure. It is realized, however, that once the cells are reconstituted with reconstitution buffer they are in a hydrated and useful form, but the combination of washings described hereinafter are preferred, specifically for clinical purposes.

After separating the cells from the reconstitution buffer by centrifugation, the resulting packed cells are preferably resuspended at room temperature in (approximately the volume used in the initial reconstitution) a wash buffer comprising nicotinic

acid, inosine, adenine, glutamine, and magnesium chloride, all present at about 0.4-10mM further comprising sodium chloride and potassium chloride each at about 30mM, buffered by 10mM disodium phosphate to pH 7.2. This wash buffer further comprises a monosaccharide, preferably glucose at a concentration of about 20mM, and a polymer, preferably polyvinylpyrroltidone, of a molecular weight 40K and present at a concentration of about 16% weight by volume. Separation by centrifugation completes the first post-rehydration step, a washing step.

After the washing step the rehydrated cells may be suspended in a dextrose-saline transfusion buffer at room temperature which preferably contains polyvinylpyrrolidone at a 10% weight by volume final concentration, with an average 2.5K molecular weight. The cells can be used as is or be returned to autologous plasma. Additional wash steps in a phosphate-buffered diluent buffer can further remove viruses, but this step is optional for preparation of rehydrated, transfusible cells.

The reconstitution and washings described above will in most instances achieve about 4 log reduction of any viral and bacterial contamination, where 1 log reduction is achieved by drying and 3 log reduction is achieved by washing. Of course, different viruses may respond differently, potentially resulting in more than 4 log reduction of contamination.

The reconstituted cells have characteristics which render them transfusable and useful for therapeutic

purposes in that their properties are similar to that of fresh (i.e. not previously lyophilized) red blood cells. Typically reconstituted red blood cells according to the present invention have an oxyhemoglobin content greater than about 90% of that in normal red blood cells. Hemoglobin recovery prior to any washing step is typically in the range of 80 to 85%. The overall cellular hemoglobin recovery including the post-hydration washing steps is about 20 to 30%. The morphology of the reconstituted cells according to the present invention (by scanning electron microscope) typically shows no holes or gaps, and primarily discocytic with some stomatocytic morphology. The oxygen carrying capacity of fresh red blood cells (as measured by P_{50}, the oxygen partial pressure at which 50% of the oxygen molecules are bound) was measured to be in the range of about 26 to 28 (average 26.7); with an average Hill coefficient (a measure of the cooperative binding of oxygen molecules to native hemoglobin) of 1.95. The typical P_{50} for erythrocytes lyophilized and reconstituted according to the present invention is about 27.5 (average) with an average Hill coefficient of 2.08. Assays of ATP in the reconstituted cells indicate ATP levels suggesting normal ATP to ADP metabolism. Normal hemagglutination by readily available blood typing antisera of red blood cells made according to the present invention is also typically found.

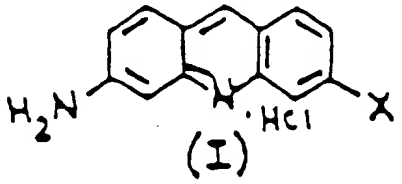
This lyophilization and reconstitution procedure advantageously and significantly diminishes viral/bacterial contamination in cell-like material (such as hemosomes), and protein fractions. The contamination can be further reduced by the radiation

sensitizing and treatment, particularly while the cells or protein fractions are in the dry state.

The starting packed red blood cells or proteins (which may initially be in a liquid or lyophilized state) are mixed with a sufficient amount (based on total wet weight of cells) of a chemical sensitizer. Preferably, in a composition of packed red blood cells (about 10% hematocrit) about 0.1 to 1 mg of the chemical sensitizer will be used per ml of packed cells. Preferably, the mixture will be irradiated with gamma radiation in the range of 3K-50K rads, typically about 3K rads. Preferred exposure is from 1-10 minutes, if using gamma radiation. Alternatively, UV light (320 nm) may be used, particularly for protein fractions. Preferred exposure is from 1-10 minutes, preferably 3 minutes, if using UV radiation. By this irradiation in presence of a sensitizer, there will be about a 6 log reduction of viral and bacterial contamination, based on contamination present prior to washing and irradiation.

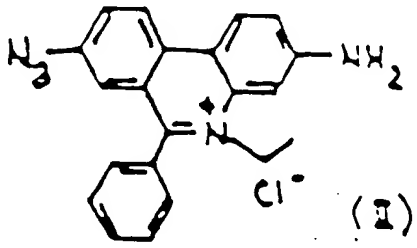
The present invention provides a selective method of generating free radicals derived from chemical sensitizers only in the vicinity of viral RNA or DNA. Indiscriminate radiolysis of blood containing virus in a hydrated state produces hydroxyl radical. However, the hydroxyl radical will damage both the red blood cells and associated proteins as well as the viral target. Thus, viral inactivation would be achieved at the sacrifice of red cell viability. Therefore, sensitizers which bind to DNA and/or RNA and which can be selected to generate radicals upon irradiation, are required. Since the radiolysis can

be performed in the dry state (preferably less than 10% residual moisture), generation of hydroxyl radicals from water is greatly reduced. In this manner indiscriminate radical damage is further prevented. Exemplary compounds include:



(I)

x = $-N_3$, -I



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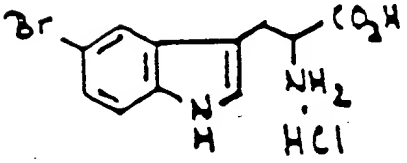

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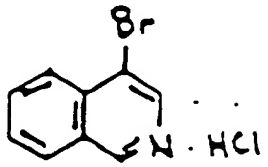

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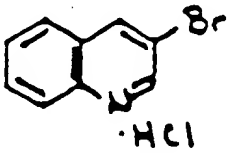


(V)

The preparations of these compounds are known. See Martin, R.F. and Kelly, D.P., Aust. J. Chem., 32, 2637-46 (1979); Firth, W., and Yielding, L.W., J. Org. Chem., 47, 3002 (1982). Other radical-generating reagents which generate radicals upon irradiation are disclosed by Platz et al., Proc. SPIE-Int. Soc. Opt. Eng. 847, 57-60 (1988) and Kanakarajan et al., JACS 110 6536-41 (1988).

The radiation-sensitizing compound (which may also be modified to bear a metal atom substituent) may also be selected from the class consisting of DNA-binding drugs, including, but not limited to, netropsin, BD peptide (a consensus peptide from HMG-1), S2 peptide, and the like. These and other DNA-binding drugs are

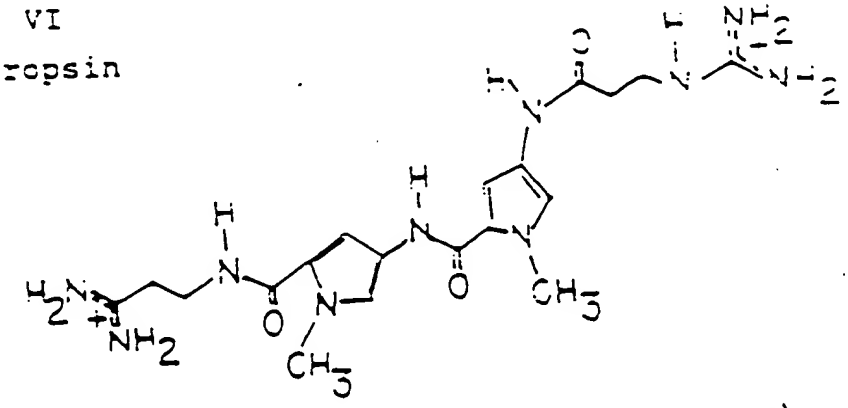
disclosed in Pjura, P.E., Grzeskowiak, K. and Dickerson, R.E. (1987), J. Mol. Biol. 197, 257-271; and Tengi, M., Usman, N., Frederick, C.A. and Wang, A.H.J. (1988), Nucleic Acids Res. 16, 2671-2690.

The radiation sensitizing compound (which may also bear a metal atom) can also comprise a class of DNA-binding proteins and/or polypeptides and/or peptides. Examples of this class of DNA-binding proteins and/or polypeptides and/or peptides are disclosed in Churchill, M.E.A. and Travers, A.A. (1991) Trends in Biochemical Sciences 16, 92-97. Specific examples of DNA-binding peptides include the SE peptide and BD peptide disclosed in the reference herein.

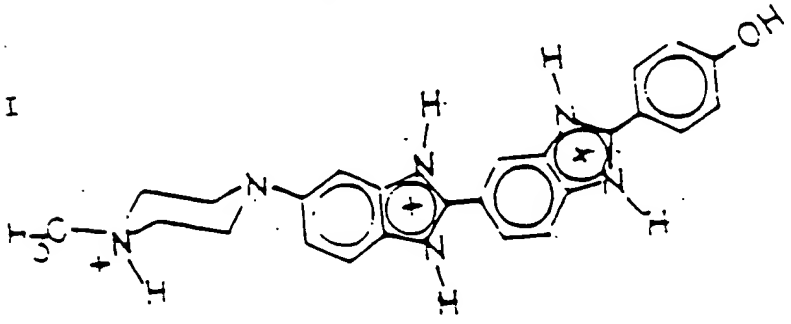
The DNA-binding specificity can be achieved by covalently coupling the radiation sensitizing compound and/or metal atom to either a DNA-binding drug or to a DNA-binding protein or polypeptide or peptide.

VI

Netropsin



VII



Other sensitizers include specially designed molecules which form triplex DNA, such as those disclosed by Youngquist and Dervan PNAS 82 2565 (1985); Van Dyke and Dervan, Science 225 1122 (1984); Van Dyke and Dervan, Nuc. Acids Res. 11 5555 (1983); Barton and Raphael, PNAS 82 6460 (1985); Barton et al., JACS 106 2172 (1984); and Barton, PNAS 81 1961 (1984). These molecules bind to DNA and RNA, site specifically, if desired, and carry reactive moieties which can generate free radicals in the proximity of the DNA or RNA.

$$R\text{-}I + e^- \rightarrow R \cdot + I^-$$

$$R\text{-}I^{-\bullet} + \text{Guanine} \rightarrow R\text{-}I + \text{Guanine}^{+\bullet}$$

While not intending to be bound by a theory, it is believed that the ejected electron will be captured by that site with the most favorable electron affinity, which is most likely a second molecule of sensitizer elsewhere in the sample. Electron capture by R-I (or R-Br) leads to dissociation of RX with the formation of a radical. The radical so generated will abstract a C-H hydrogen atom from a sugar moiety of a nearby nucleic acid which in turn will lead to DNA or RNA cleavage and viral inactivation.

The radical cation of the sensitizer ($R\text{-}X^{-\bullet}$) will eventually abstract an electron from that component of the sample with the most favorable oxidation potential. This is most likely guanine. The electron transfer reaction forms guanine radical cation. This substance will react with O_2 upon reconstitution with aerated H_2O. This process also leads to DNA cleavage and viral inactivation. Unreacted material and reaction by-products will be

removed during the washing steps involved in the reconstitution of the lyophilized cells (Table 2). This process will also further remove any virus not inactivated by the treatment described above.

Compounds (1) and (2) bind tightly to DNA and RNA by either intercalation and/or by electrostatic interactions between positively charged ammonium ion groups and the negatively charged phosphate groups of the nucleic acid target. Red blood cells do not contain nucleic acids and accordingly will not bind to such compounds by intercalation.

The best mode for using the invention is to add the sensitizer to potentially contaminated blood solutions, and to expose to gamma radiation or x-rays. Fluid solutions of blood are preferably exposed to 3000 rads, and dried lyophilized solid formulations are preferably exposed to 10,000 rads of radiation. It is known that the red cells will survive these doses of radiation in the absence of a sensitizer. Lyophilized blood can withstand higher dosage levels of radiation than hydrated blood.

The gamma radiation or x-ray will be absorbed primarily by the heavy atom of the sensitizer, which will be bound to viral DNA or RNA. The radiation will ionize the sensitizer as follows:

$$R\text{-}I + \gamma\text{-Ray} \text{ (X-Ray)} \rightarrow R\text{-}I^{+\bullet} + e^{-}$$

In some instances, particularly if the sensitizer and red blood cells are allowed to stand together for more than several minutes, sensitizers may diffuse into the red blood cells prior to lyophilization.

Antioxidants such as glutathione (an excellent hydrogen atom donor) may be added to the preparation to augment the red cell defenses against free radical initiated damage. It will be understood that incorporation of the sensitizer into cells will also allow inactivation of intracellular viruses, especially viruses thought to reside inside white blood cells (most packed red blood cell units contain residual white cells), or intracellular blood parasites, such as malaria parasite which infects red blood cells.

The sensitizers are removed from the reconstituted blood serum or protein fraction by the washing protocol described above for lyophilized cells.

It is preferred that gamma or X-ray radiolysis take place in a dried lyophilized blood (or protein), virus, and sensitizer formulation rather than in a wet, fluid material for several reasons. Firstly, the dry material is less sensitive to radiation and can be exposed to larger doses of γ-rays or other penetrating radiation without damage to red blood cells (Table 1). This increases the extent of radiolysis of the sensitizer. Secondly, sensitizer radicals bound to DNA or RNA in the dry state can not dissociate from the virus due to the lack of diffusion in the solid material. This will force the sensitizer radical to react with viral RNA or DNA. Thirdly, the solid state conditions will enhance hydrogen atom transfer reactions of the sensitizer radical with the viral nucleic acid, perhaps by quantum mechanical tunneling. Fourthly, the reconstitution and washing protocol used with lyophilized blood or protein fraction serves as a

means to remove unreacted material or reaction by-products, and further removes any virus not affected by the treatment (Table 2).

Other types of radiation may be used including ionizing radiation in general, such as X-ray radiation. In one embodiment a metal atom may be a substituent on a chemical radiation sensitizer molecule which binds to nucleic acids, thereby targeting the embodiments such as bacteria, parasites and viruses. Metal atom substituents of chemical sensitizers for this purpose include Br, I, Zn, Cl, Ca and F. The X-ray source is preferably a tunable source, so that the radiation may be confined to a narrow wavelength and energy band, if so desired. The tunable feature allows for optimization of energy absorption by the metal atoms, thereby directing the absorbed penetrating radiation energy to the production of radicals by a chemical sensitizer bound to nucleic acid.

The present invention is applicable to contaminants which comprise single or double-stranded nucleic acid chains, including RNA and DNA, and viruses, bacteria or other parasites comprising RNA and/or DNA.

To illustrate the invention, red blood cells were lyophilized as described above, irradiated, and tested for erythrocyte characteristics measured. The results are shown in Table 1. The same procedure was then used, except that the bacteriophage T4 (in dextrose saline) was mixed with the cells and then washed successively with four different wash buffers. The results are shown in Table 2.

Table 1: Influence of irradiation on lyophilized reconstituted red blood cells. Doses as high as 20,000-50,000 rads do not affect cells in the dry state according to the parameters assayed after reconstitution and listed below.

Exposure of Lyophilized Cells to Gamma Irradiation

	* Percentage of Control	
Dosage Level	20,000 rads	50,000 rads
Hb Recovery	100	99
Oxy Hb	No Change from starting value	No Change from starting value
Cell Indices		
MCV	99	98
MCH	100	100
MCHC	100	100
Metabolism		
ATP (μmol/g Hb)	79	79
Lactate (μmol/g Hb/Hr)	86	79

* Control cells were non-irradiated, lyophilized reconstituted cells.

Table 2: Reduction in viral titre as a function of washing of the red cells. The procedure used in reconstituting the lyophilized cells involves several washing steps which also reduce the viral titre. The extent of reduction with each wash decreases until a practical limit is attained. This represents an approximate 4 log reduction in viral titre.

Washing Protocol Reduction of Viral Load in Blood

Buffer Wash Step	Total Amount of Virus	Log Reduction
Experiment 1 (non-lyophilized cells)	-	
Reconstitution	$7.3 \text{ Ox } 10^7$	0
Wash	4.80×10^4	3.2
Diluent	2.08×10^4	3.5
Transfusion	3.50×10^4	3.3
Experiment 2 (lyophilized cells)		
Lyophilization	3.68 x 108	0
Reconstitution	2.11 x 107	1.2**
Wash	2.38 x 104	4.2
Diluent	2.00 x 104	4.3
Transfusion	4.06 x 104	4.0

In Experiment 1, the effects of lyophilization on viral reduction are not included. In Experiment 2, these effects are included. The marker virus used in these cases was bacteriophage T4. The extent of reduction was determined using the plaque assay.

**This shows an additional about 1 log reduction of contamination due to the drying step.

EXAMPLE 1

Packed human red blood cells purified from donated whole blood are washed free of the anticoagulant storage solution (commercially available CPDA, containing citrate/phosphate/dextrose/adenine), and suspended in dextrose-saline at a 10% hematocrit. Approximately 10 ml of washed packed red cells is placed in a quartz chamber and exposed to U.V. light, preferably at 320 nm, for 2 minute time intervals, up to a 10 minute total exposure. At each 2 minute interval the suspension is mixed and a small sample of red cells (10 microliters) is removed and diluted into 2 ml of water for spectrophotometric assay of hemoglobin. At each step the temperature of the irradiated red cell suspension is measured, to ensure that the suspension did not overheat. At no point did the suspension exceed 26 degrees C (normal body temperature is 37 degrees C). Untreated red cells contain a high proportion of functional oxyhemoglobin (oxyHb), usually in the range of 96% or higher. Oxidation damage can form a semi-stable methemoglobin species (metHb), which can normally be reduced back to oxyhemoglobin by a cellular repair enzyme. Hemichrome represents a more severely damaged form, and can be irreversible. Normal red cells can tolerate a moderate level of methemoglobin. Hemichrome degradation can produce free heme, the iron-porphyrin component of native hemoglobin, which is damaging to cell membranes. Thus it is desirable to minimize hemichrome levels. Each hemoglobin species can be detected at a specific wavelength, using a standard spectrophotometer.

The following data show the sensitivity of the hemoglobin to damage by the increased U.V. exposure.

An exposure of 3 minutes was judged to be usable for viral inactivation using a radiation sensitizer, without inflicting excessive damage to red blood cells.

EXPOSURE (Minutes)	% OXYHB	% METHB	% HEMI
0	96.6	3.4	0
2	90.2	7.5	2.3
4	84.5	13.4	2.1
6	76.7	22.5	0.9
8	72.6	27.4	0
10	66.4	33.6	0

EXAMPLE 2

A suspension (0.1 ml) of bacteriophage lambda or bacteriophage phi-X174, of at least 10EV PFU/ml, is separately added to 4 ml of dextrose-saline containing 1 mg/ml of compounds I or II or III. Each suspension of bacteriophage with a radiation sensitizing compound is then exposed to U.V. radiation of the preferred wavelength (320 nm) in a quartz chamber for the preferred time (3 minutes). A control sample of each bacteriophage suspension, containing a sensitizer, is not exposed to U.V. light. Serial dilutions are performed to quantitate the level of infectious titer, and aliquots of the various bacteriophage samples are then mixed with host bacteria and spread on nutrient agar. Following a normal growth period, the plates are assayed for plaques. Other bacteriophage suspensions are separately irradiated as above, but without added sensitizer, to demonstrate the effect of this dose of U.V. alone.

COMPOUND	Log10 Reduction of Virus Titer	
	phi-X174	Lambda
I ($X=N_3$)	>6.0	>6.0
I (X=I)	4.0	>6.0
II	1.7	>6.0
No compound	2-3	2-3

From these data it can be seen that all three tested compounds significantly increase the sensitivity of double-stranded DNA virus (lambda) to U.V. of the preferred exposure. Compound I is also effective against a single-stranded DNA virus, phi-X174. Compound I is most preferred, showing a high (at least 6 log reduction) inactivation efficacy against both single-strand and double-strand DNA viruses.

WHAT IS CLAIMED IS:

1. A process of reducing viral and/or bacterial contamination in a dried or reconstituted composition comprising red blood cells, platelets, and/or proteins comprising:

mixing said composition with a sufficient volume of a phosphate-buffered reconstitution solution to form a mixture, wherein said reconstitution solution has a pH in the range of about 7.0-7.4 at a temperature in the range of about 15-50°C, said reconstitution solution further comprising a final concentration of about 0.7% by weight up to the saturation concentration of a polymer or mixture of polymers having a molecular weight in the range of about 1K to 360K,

separating said red blood cells, platelets and/or proteins from said mixture by centrifugation and washing by at least one wash cycle by resuspending said red blood cells, platelets and/or proteins in a dextrose-polymer wash buffer solution at a pH in the range of about 7.0-7.4 and separating by centrifugation to produce substantially decontaminated red blood cells, platelets and/or proteins.

2. A process according to Claim 1 further comprising the step of freeze-drying said decontaminated red blood cells, platelets and/or proteins.

3. A process according to Claim 1 or 2 wherein said polymers are amphipathic.

4. A process according to Claim 1 or 2 wherein said polymers have a molecular weight in the range of about 2.5K to 500K.

5. The process of Claim 1 or 2 wherein said composition further comprises a monosaccharide selected from the group consisting of xylose, glucose, ribose, mannose and fructose.

6. The process of Claim 1 or 2 wherein said mixture of polymers comprises polyvinylpyrrolidone and hydroxyethyl starch.

7. The process of Claim 5 wherein said polymer comprises polyvinylpyrrolidone.

8. The process of Claim 7 wherein said polyvinylpyrrolidone has an average molecular weight of about 10K.

9. The process of claim 7 wherein said polyvinylpyrrolidone has an average molecular weight of about 40K.

10. The process of Claim 7 wherein said polyvinylpyrrolidone has an average molecular weight of about 360K.

11. The process according to Claim 6 wherein said hydroxyethyl starch has an average molecular weight of about 500K.

12. A process according to Claim 1 further comprising the steps of contacting said composition with at least one chemical sensitizer selected from

the group consisting of compounds which bind to DNA and/or RNA and are capable of selectively generating free radicals upon exposure to radiation, and exposing said composition to radiation of sufficient wavelength and intensity for a period of time sufficient to cause said sensitizer to further reduce viral and bacterial contamination in said composition.

13. A process according to Claim 12 wherein said composition comprises red blood cells.

14. A process according to Claim 12 wherein said composition comprises platelets.

15. A process according to Claim 12 wherein said composition comprises blood plasma proteins.

16. A process according to Claim 12 wherein said radiation comprises gamma radiation.

17. A process according to Claim 12 wherein said contamination comprises single- and/or double-stranded-type viruses.

18. A process for reducing viral and/or bacterial contaminations in a protein composition comprising the steps of contacting said composition with at least one chemical sensitizer selected from the group consisting of compounds which bind to DNA and/or RNA and are capable of selectively generating free radicals upon exposure to radiation, and exposing said composition to radiation of sufficient wavelength and intensity for a period of time sufficient to cause said sensitizer to further reduce

viral and bacterial contamination in said composition.

19. A process according to Claim 18 wherein said composition comprises blood proteins.

20. A process according to Claim 19 wherein said composition is in a lyophilized form.

21. A process according to Claim 19 wherein said composition is a blood protein liquid fraction.

22. A process according to Claim 19 wherein said composition comprises immune globulins.

23. A process according to Claim 19 wherein said composition comprises blood serum albumin.

24. A process according to Claim 19 wherein said composition comprises a clotting factor.

25. A process according to Claim 24 wherein said clotting factor comprises Factor VIII.

26. A process according to Claim 24 wherein said clotting factor comprises Factor IX.

27. A process according to Claim 18 wherein said radiation comprises gamma radiation.

28. A process according to Claim 18 wherein said contamination comprises single- and/or double-stranded-type viruses

29. A substantially virally and bacterially decontaminated lyophilized composition comprising red blood cells, platelets and/or proteins, said decontamination resulting from exposure to electromagnetic radiation of sufficient wavelength and intensity to inactivate viral and bacterial contamination in said composition.

30. A substantially virally and bacterially decontaminated lyophilized composition comprising red blood cells, platelets and/or proteins and containing inactive viral and/or bacterial contaminants which have been deactivated by binding of the viral and/or bacterial DNA or RNA to at least one chemical sensitizer capable of selectively generating free radicals upon exposure to electromagnetic radiation, and by exposing said bound sensitizer to electromagnetic radiation of sufficient wavelength and intensity and for a period of time sufficient to cause said sensitizer to deactivate said RNA and/or DNA.

31. A composition according to Claim 29 or 30 comprising platelets.

32. A composition according to Claim 29 or 30 comprising red blood cells.

33. A composition according to Claim 29 or 30 comprising blood proteins.

34. A composition according to Claim 33 comprising a clotting factor.

35. A composition according to Claim 33 comprising plasma proteins.

36. A composition according to Claim 33 comprising blood protein extracts.

37. A composition according to Claim 34 comprising Factor VIII.

38. A composition according to Claim 34 comprising Factor IX.

39. A rehydrated composition formed by rehydration of the composition of Claim 29 or 30.

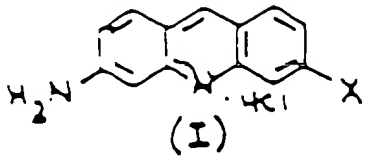
40. A rehydrated and washed composition formed by washing the composition according to Claim 39.

41. A composition according to Claim 33 comprising immune globulins.

42. A composition according to Claim 33 comprising serum albumin.

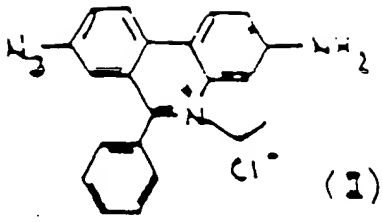
43. A method of reducing viral and/or bacterial contamination in dried or reconstituted blood cellular matter comprising red blood cells, and/or platelets, comprising the step of contracting said blood cellular matter with at least one chemical sensitizer selected from the group consisting of

compounds of the formulas:

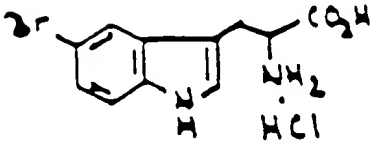


(I)

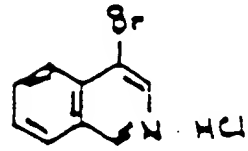
x = $-N_3$, -I



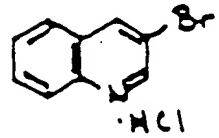
(II)



(III)



(IV)



(V)

exposing said cellular matter to radiation of sufficient wavelength and intensity for a period of time sufficient to cause said sensitizer to substantially reduce viral and bacterial contamination in said blood cellular matter.

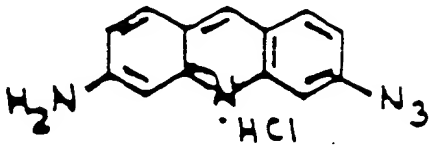
44. A method according to Claim 43 wherein said cellular matter comprises erythrocytes.

45. A method according to Claim 43 wherein said cellular matter comprises platelets.

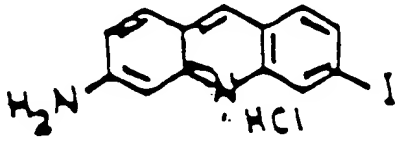
46. A method according to Claim 43 wherein said radiation comprises ultraviolet radiation.

47. A method according to Claim 43 wherein said contamination comprises single- and/or double-stranded-type viruses.

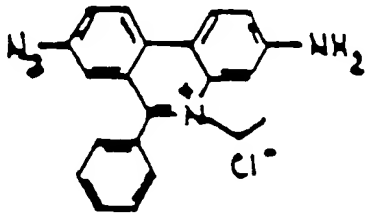
48. A method according to Claim 43 wherein said sensitizer comprises a compound of the formula



49. A method according to Claim 43 wherein said sensitizer comprises a compound of the formula



50. A method according to Claim 43 wherein said sensitizer comprises a compound of the formula



51. A process for reducing viral and/or bacterial and/or parasitic contamination in dried or reconstituted blood matter comprising red blood cells, plasma proteins and/or platelets, comprising the step of contacting said blood matter with at least one radiation-sensitizing compound.

52. A process according to Claim 1 wherein said radiation-sensitizing compound comprises a nucleic acid-binding compound.

53. A process according to Claim 1 or 2 wherein said radiation sensitizing compound comprises a metal atom.

54. A process according to Claim 53 wherein said metal atom comprises Br.

55. A process according to Claim 53 wherein said metal atom comprises I.

56. A process according to Claim 53 wherein said metal atom comprises Zn.

57. A process according to Claim 53 wherein said metal atom comprises Cl.

58. A process according to Claim 53 wherein said metal atom comprises Ca.

59. A process according to Claim 53 wherein said metal atom comprises F.

60. A method according to Claim 51 wherein said compound is sensitized by penetrating, ionizing radiation.

61. A method according to Claim 51 wherein said compound is sensitized by gamma radiation or X-rays.

62. A method according to Claim 52 wherein said radiation-sensitizing compound binds RNA.

63. A method according to Claim 52 wherein said radiation-sensitizing compound binds DNA.

64. A method according to Claim 51 wherein said contamination comprises RNA- and/or DNA-containing viruses.

65. A method according to Claim 51 wherein said contamination comprises RNA- and/or DNA-containing bacteria.

66. A method according to Claim 51 wherein said contamination comprises RNA- and/or DNA-containing parasites.

67. A method according to Claim 53 wherein said compound is sensitized by tuned, penetrating, ionizing radiation from a tunable radiation source capable of emitting selected wavelengths, frequencies, and/or intensities of radiation.

68. A method according to Claim 67 wherein said tuned ionizing radiation comprises gamma-wavelength radiation.

69. A method according to Claim 67 wherein said tuned ionizing radiation comprises X-ray radiation.

70. A method according to Claim 67 wherein said tuned ionizing radiation is tuned to select particular wavelength, frequency and/or intensity ranges to optimize absorption of the radiation energy by said metal atom substituent of said radiation sensitizing compound.

71. A method according to Claim 51 wherein said radiation-sensitizing compound is coupled to a

monoclonal antibody or polyclonal antibodies directed against viral, bacterial and/or parasitic antigens.

72. A method according to Claim 71 wherein said radiation-sensitizing compound comprises a metal atom.

73. A method according to Claims 71 or 72 wherein said radiation sensitizing compound is activated by penetrating, ionizing radiation.

74. A method according to Claim 73 wherein said radiation comprises gamma radiation or X-rays.

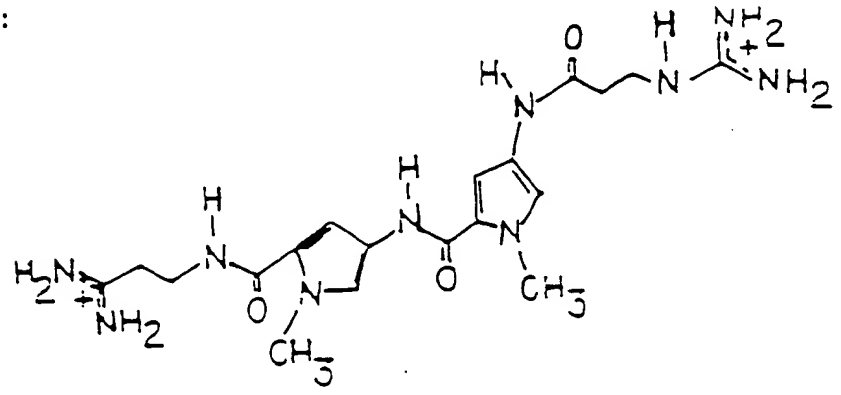
75. A method according to Claim 71 wherein said antigens comprise viral surface epitopes or viral envelope proteins.

76. A method according to Claim 71 wherein said antigens comprise bacterial surface epitopes.

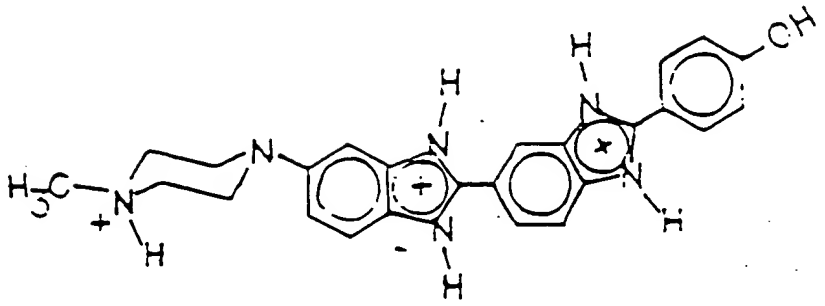
77. A method according to Claim 71 wherein said antigens comprise surface epitopes of blood-transmitted parasites.

78. A method according to Claim 12 or 18 wherein said sensitizer comprises DNA-binding drugs.

79. A method according to Claim 78 wherein said DNA-binding drug comprises a compound of the formula VI:



80. A method according to Claim 78 wherein said DNA-binding drug comprises a component of the formula VII:



81. A method according to Claim 78 wherein said DNA-binding drug contains a metal atom substituent.

82. A method according to Claim 78 wherein said DNA-binding radiation sensitizer is activated by ionizing penetrating radiation.

83. A method according to Claim 82 wherein said ionizing radiation comprises gamma radiation or X-rays.

84. A method according to Claim 12 or 18 wherein said sensitizer comprises a DNA-binding protein, polypeptide, and/or peptide.

85. A method according to Claim 84 wherein said peptide is S2 peptide or BD peptide.

86. A method according to Claim 84 wherein said DNA-binding protein, polypeptide, or peptide contains a metal atom substituent.

87. A method according to Claim 84 wherein said DNA-binding protein, polypeptide, and/or peptide is activated by ionizing penetrating radiation.

88. A method according to Claim 87 wherein said ionizing radiation comprises gamma radiation or X-rays.

89. A composition comprising cellular blood matter or blood proteins in which viral, bacterial, or parasitic contaminants have been substantially inactivated according to the method of Claim 78.

90. A composition comprising cellular blood matter or blood proteins in which viral, bacterial, or parasitic contaminants have been substantially inactivated according to the method of Claim 84.

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 35/14
U.S. CL.: 435/2; 424/529, 530, 531, 532, 533, 534

II. FIELDS SEARCHED

Minimum Documentation Searched *

Classification System	Classification Symbols
U.S.	435/2; 424/529, 530, 531, 532, 533, 534

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *

APS, CA Reg, CAS, BIOSIS

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No. **
Y	Vox Sang, Vol. 26, issued 1974 K. GANSHIRT ET AL., "A five-bag system for washing fresh and frozen erythrocytes and their preservation", pages 66-73, see page 66.	1-17
Y	Cryobiology, Vol. 10, issued 1973, D. PRIBOR, "Studies with Dextran 40 in cryopreservation of blood", pages 93-103, see entire article.	1-17
Y	Acta Vet Scand, Vol. 20, issued 1979, V. MYHRVOID, "Cryopreservation of sheep red blood cells", pages 531-536, see entire article.	1-17
Y	US, A, 4,874,690 (GOODRICH ET AL), 17 October 1989, see entire document.	1-17
X	US, A, 4,071,412 (EISENBERG ET AL.) 31 January 1978, see entire document.	1-17

(cont.)

* Special categories of cited documents: **

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
09 July 1991	12 AUG 1991

International Searching Authority	Authorized Officer
ISA/US	Sandra Saucier (vsh)

Form PCT/ISA/210 (second sheet) (July 1989)

(CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X Y	US, A, 4,878,891 (JUDY ET AL.) 07 November 1989, see the entire document.	18,19,21-26,28 39,41,42,71, 75-78,89,90
X Y	Mutation Research, Vol. 81, issued 1981, W. FIRTH ET AL., "Azido Analogs of Acridine: PHOTOAFFINITY PROBES FOR FRAMESHIFT MUTAGENESIS IN SALMONELLA typhimurium", pages 295-309, see page 299.	43 — 48
Y	R. ACHESON ET AL., "ACRIDINES" published 1956 by Interscience Publishers, Inc. (N.Y.), pages 339-361, see pages 352-355.	43-47,49
Y,P	US, A, 4,950,665 (FLOYD) 21 August 1990, see entire document.	51,52,62-65
X	US, A, 4,409,105 (HAYASHI ET AL.) 11 October 1983, see col. 7, ln. 30.	29,33,35,36, 39,51,60,61, 67,68,70,89, 90
X	VOX SANG., Vol. 55, issued 1988, G. ESPERSEN ET AL., "IRRADIATED BLOOD PLATELET CONCENTRATES STORED FOR FIVE DAYS - EVALUATION BY IN VITRO TESTS", pages 218-221, see abstract.	89, 90
Y	EXP. PARASITOL., Vol. 31, issued 1972, C. LANTZ ET AL., "PLASMODIUM berglei: INHIBITED INCORPORATION OF AMP-8-3H into NUCLEIC ACIDS OF ERYTHROCYTE-FREE MALARIAL PARASITES BY ACRIDINES, PHENANTHRIDINES, AND 8-AMINOQUINOLINES", pages 255-261, see page 258.	43-46 51-53,57
Y	PHARM. DELT., EPISTOM. EKDOSIS, Vol. 1, No. 2, issued 1971, J. POLAK ET AL. "THE BACTERICIDAL AND FUNGICIDAL ACTIVITY OF SOME QUINOLINIUM COMPOUNDS", pages 27-33, see entire article.	43-47
Y	US, A, 4,684,521 (EDELSON) 04 August 1987, see abstract.	43-47,50,51, 62-66,71,72, 75-77,81,84, 86

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [2] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [3], specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

(See Attached Sheet)

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest and, where applicable, the payment of a protest fee.

☒ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (second sheet) (July 1992)

I. Claims 1-11: A method of decontaminating red blood cells by washing, Class 435, subclass 2.

II. Claims 12-28, 78, 81-88:

A method of sterilizing a protein composition with the use of a chemical sensitizer, Class 435, subclass 2.

III. Claim 29: A composition sterilized by electromagnetic radiation, Class 435, subclass 2.

IV. Claims 30-42 & 89-90:

A composition sterilized by use of a chemical sensitizer, Class 435, subclass 2.

V. Claim 43 (I): A method of sterilizing blood cells with a chemical sterilizer, Class 435, subclass 2.

VI. Claim 43 (II): A method of sterilizing blood cells with a chemical sterilizer, Class 435, subclass 2.

VII. Claim 43 (III): A method of sterilizing blood cells with a chemical sterilizer, Class 435, subclass 2.

VIII. Claim 43 (IV): A method of sterilizing blood cells with a chemical sterilizer, Class 435 subclass 2.

IX. Claim 43 (V): A method of sterilizing blood cells with a chemical sterilizer, Class 435, subclass 2.

Claims 44-50 will be examined to the extent that they read upon the inventions of Groups V-IX.

X. Claims 51-53: A method of sterilizing blood cells with a chemical sterilizer, Class 435, subclass 2.

XI. Claim 54: A method of sterilizing blood cells with a chemical sterilizer, Class 435, subclass 2.

XII. Claim 55: A method of sterilizing blood cells with a chemical sterilizer, Class 435, subclass 2.

XIII. Claim 56: A method of sterilizing blood cells with a chemical sterilizer, Class 435, subclass 2.

XIV. Claim 57: A method of sterilizing blood cells with a chemical sterilizer, Class 435, subclass 2.

XV. Claim 58: A method of sterilizing blood cells with a chemical sterilizer, Class 435, subclass 2.

XVI. Claim 59: A method of sterilizing blood cells with a chemical sterilizer, Class 435, subclass 2.

Claims 60-70 will be examined to the extent that they read upon the invention of Group XI-XVI.

XVII. Claims 71-74 & 84-88:

A method of sterilizing blood cells with a chemical sterilizer attached to an antibody, Class 435, subclass 2.

XVIII. Claim 75: A method of sterilizing blood cells with a chemical sterilizer attached to an antibody, Class 435, subclass 2.

IXX. Claim 76: A method of sterilizing blood cells with a chemical sterilizer attached to an antibody, Class 435, subclass 2.

XX. Claim 77: A method of sterilizing blood cells with a chemical sterilizer attached to an antibody, Class 435, subclass 2.

XXI. Claim 79: A method of sterilizing blood cells with a chemical sterilizer. Class 435, subclass 2.

XXII. Claim 80: A method of sterilizing blood cells with a chemical sterilizer, Class 435, subclass 2.

The claims of these groups, although all drawn to sterilization methods for blood components and therefore, classified in the similar class and subclasses vary in scope and chemical structure from each other. This necessitates different literature searches, because the search for one chemical sensitizer would not encompass the search for a chemical sensitizer of another group. PCT Rules 13.1 and 13.2 do not provide for multiple distinct methods within a general inventive concept.